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Abstract: Heterostyly is a genetically determined floral polymorphism of style length promoting outcrossing between individuals of different morphs, which usually coexist within populations at equal frequencies. Loss in the area and connectivity of suitable habitats may cause deviations from the expected equal morph frequencies. However, there is a need to evaluate the generality of this pattern at larger spatial extents and to identify possible underlying mechanisms. A citizen-science approach was used to study morph frequencies in populations of the heterostylous grassland plant *Primula veris* across Estonia. We developed an online platform to facilitate an easy upload of the data. We examined the effect of the following variables in the surroundings of the study populations reflecting the landscape context on the deviation of morph ratios: (a) semi-natural grasslands, (b) forests and shrubs, (c) human population density and (d) a proxy for plant population size. The citizen-science approach provided unprecedented density of data from 1,700 localities. Nearly half of these observations, which were maintained for further analysis after data filtering, included over 62,000 short-styled morphs and about 54,000 long-styled morphs. Small populations were characterized by higher overall deviation of morph ratios from isoplethy (equal morph ratio). Deviation increased in semi-natural grasslands located in regions with high human population density. The significant effect of human population density and plant population size on deviations of morph frequencies suggests the role of stochastic demographic effects of habitat fragmentation on morph ratios. Overall lower proportion of long-styled morphs indicates that partial intra-morph compatibility shown in long-styled morphs may lead to higher inbreeding and related decline in fitness and abundance. **Synthesis.** Citizen-science data about the morph type of *Primula veris* across Estonia obtained with the help of thousands of people demonstrates that in addition to plant population size, landscape context may affect plant reproductive traits, such as heterostyly. Larger population size of *P. veris* can help to buffer against random fluctuations in this trait. Increasing impact of human activities may have a negative impact on both small and large populations. The exact underlying mechanisms of the prevalence of one morph over the other, however, pose novel questions for further research.

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Landscape context and plant population size affect morph frequencies in heterostylous *Primula veris*—Results of a nationwide citizen-science campaign

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Abstract

1. Heterostyly is a genetically determined floral polymorphism of style length promoting outcrossing between individuals of different morphs, which usually coexist within populations at equal frequencies. Loss in the area and connectivity of suitable habitats may cause deviations from the expected equal morph frequencies. However, there is a need to evaluate the generality of this pattern at larger spatial extents and to identify possible underlying mechanisms.
2. A citizen-science approach was used to study morph frequencies in populations of the heterostylous grassland plant *Primula veris* across Estonia. We developed an on-line platform to facilitate an easy upload of the data. We examined the effect of the following variables in the surroundings of the study populations reflecting the landscape context on the deviation of morph ratios: (a) semi-natural grasslands, (b) forests and shrubs, (c) human population density and (d) a proxy for plant population size.
3. The citizen-science approach provided unprecedented density of data from 1,700 localities. Nearly half of these observations, which were maintained for further analysis after data filtering, included over 62,000 short-styled morphs and about 54,000 long-styled morphs. Small populations were characterized by higher overall deviation of morph ratios from isoplethy (equal morph ratio). Deviation increased in semi-natural grasslands located in regions with high human population density.
4. The significant effect of human population density and plant population size on deviations of morph frequencies suggests the role of stochastic demographic effects of habitat fragmentation on morph ratios. Overall lower proportion of long-styled morphs indicates that partial intra-morph compatibility shown in long-styled morphs may lead to higher inbreeding and related decline in fitness and abundance.
5. *Synthesis.* Citizen-science data about the morph type of *Primula veris* across Estonia obtained with the help of thousands of people demonstrates that in addition to plant

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population size, landscape context may affect plant reproductive traits, such as heterostyly. Larger population size of *P. veris* can help to buffer against random fluctuations in this trait. Increasing impact of human activities may have a negative impact on both small and large populations. The exact underlying mechanisms of the prevalence of one morph over the other, however, pose novel questions for further research.

KEYWORDS

distyly, habitat fragmentation, heterostyly, pollination, semi-natural grasslands

1 | INTRODUCTION

European semi-natural grasslands, which have evolved and persisted over thousands of years as a result of moderate grazing and mowing, are hotspots of European biodiversity (Habel et al., 2013; WallisDeVries, Poschlod, & Willems, 2002; Wilson, Peet, Dengler, & Pärtel, 2012). In addition, they provide various important ecosystem services, such as pollination and supply of sustainably produced agricultural products (Bastian, 2013; Lindgren, Lindborg, & Cousins, 2018; Öckinger & Smith, 2007). However, degradation and isolation of grassland habitats because of land-use change during the last century have led to an unprecedented loss of grassland plants across Europe (Auffret, Kimberley, Plue, & Waldén, 2018; Cousins, Auffret, Lindgren, & Tränk, 2015; Hooftman & Bullock, 2012). The viability of remaining populations that are often small in size and spatially isolated is jeopardized by low quality of habitats due to lack of management (Aavik, Jögar, Liira, Tulva, & Zobel, 2008), eutrophication (Stevens, Dise, Mountford, & Gowing, 2004), low genetic diversity within populations and reduced gene flow among populations (Aavik & Helm, 2018; Aavik, Holderegger, Edwards, & Billeter, 2013; Honnay & Jacquemyn, 2007; Pico & van Groenendaal, 2007). All these factors are putting enormous pressure on many grassland species, which have to respond either by adapting to novel selection pressures (Bodbyl Roels & Kelly, 2011; Jacquemyn, De Meester, Jongejans, & Honnay, 2012), by migrating to more favourable environments (Ash, Givnish, & Waller, 2017) or, in the worst case, by going locally, and eventually globally, extinct.

A large majority of flowering plants, including numerous grassland plants, depend on insect-mediated pollen flow for effective reproduction (Clough et al., 2014; Jakobsson & Ågren, 2014; Ollerton, Winfree, & Tarrant, 2011). However, the survival of small and isolated plant populations is increasingly threatened by the disruption of such ecological interactions (Kohler, Verhulst, van Klink, & Kleijn, 2008; Valiente-Banuet et al., 2015). Fragmentation of semi-natural grasslands has led to a severe loss of pollinators, imposing negative consequences for pollen-mediated gene flow and related fitness of plants (Biesmeijer et al., 2006; Ollerton et al., 2011; Steffan-Dewenter & Westphal, 2008), particularly for outcrossing plants relying on pollen flow between plant individuals (Aguilar, Ashworth, Galetto, & Aizen, 2006; Estrada, Morales-Castilla, Meireles, Caplat, & Early, 2018). Heterostyly is a floral polymorphism, where populations comprise of two (or three) genetically determined morphs reciprocally

differing in style length and stamen position. This trait occurs in at least 27 plant families as an adaptation for ensuring cross-pollination (Figure 1). In distylous species, such as most primroses (*Primula*), these morphs are referred to as L-morphs/pin morphs (i.e. long-styled morphs) and S-morphs/thrum morphs (i.e. short-styled morphs). Inter-morph differences are expressed also in other floral characteristics, such as the length of stigmatic papillae and the size of pollen grains. Charles Darwin in his landmark findings about the functional meaning of distyly in *Primula veris* was the first to suggest that such floral heteromorphism serves as an effective outcrossing mechanism (Darwin, 1862). Morphological dimorphism is accompanied by a genetically determined incompatibility system (Huu et al., 2016), which ensures crossing of plants with the ones characterized by the opposite style morph and prevents mating between individuals sharing the same floral morph characteristics. The heteromorphism is governed by a supergene; it comprises a series of genes closely spaced or linked on a chromosome. Short-styled individuals were thought to be heterozygous (Ss) and long-styled homozygous recessive (ss; Li, Webster, Furuya, & Gilmartin, 2007), but recent molecular genetic analyses in *Primula* suggest that the heterostyly-locus is a hemizygous region present in short-styled morphs, but absent from long-styled morphs (Huu et al., 2016; Nowak et al., 2015).

Rapid changes in land-use intensity, related negative effects on area and connectivity of natural and semi-natural habitats, and a loss of pollinator communities have brought the consequences of landscape change for heterostylous plants into the spotlight (Brys & Jacquemyn, 2015; Jacquemyn et al., 2012; Kéry, Matthies, & Schmid, 2003). The genetically determined incompatibility system in association with floral heteromorphism leads to symmetrical disassortative mating and generally equal morph frequencies at equilibrium conditions (Barrett & Shore, 2008). However, high spatio-temporal dynamics of natural and semi-natural habitats as a result of recent land-use change may shift such equilibria and related population viability in several ways. Severe habitat loss, being exemplified by the reduced area of European semi-natural grasslands, has substantially decreased the size of many plant populations (Kiviniemi, 2008). Resulting population bottlenecks and drift effects may lead to stochastic deviation of morphs from equal frequencies (Endels, Jacquemyn, Brys, & Hermy, 2002; Kéry et al., 2003). This, in turn, lowers the abundance of suitable partners for mating and thus reduces reproductive fitness (Jacquemyn, Brys, & Hermy, 2002; Kéry et al., 2003). Nevertheless, the system of heterostyly is not completely leakage-proof (Brys &



FIGURE 1 A screenshot view from the specifically designed web-platform (<https://nurmenukk.ee/et>) for an easy upload of the data within the frames of the citizen-science campaign 'Eesti otsib nurmenukke' carried out in Estonia in 2019. The view represents the explanation of the task of discriminating between short-styled S-morphs ('S-tüüp') and long-styled L-morphs ('L-tüüp') of *Primula veris*, and the tool to insert/remove data about the corresponding morph. Translation of Estonian text: 'Determine the morph of 100 plant individuals. In case there are fewer individuals, determine the morph of all individuals. In S-morph, several anthers are visible from above, while in L-morph only a single dot representing the stigma is visible'

Jacquemyn, 2015, 2020; Wedderburn & Richards, 1990). According to Wedderburn and Richards (1990), crosses between L-morphs of *P. veris* may lead to seed set in up to 14.5% cases, while effective intra-morph fertilization between S-morphs occurs very rarely despite the disproportionate inter-morph pollen flow in favour of short-styled morphs (Ornduff, 1980). Thus, L-morphs may have an advantage in conditions of the lack of compatible pollen (Van Rossum, De Sousa, & Triest, 2006) resulting from the scarcity of mates and/or an absence of pollinators, and should consequently be prevalent in fragmented habitats with smaller populations. With that, for example, high genetic diversity has been observed in populations dominated by L-morphs in distylous *Pulmonaria officinalis*, most probably owing to reproductive advantages of L-morphs in this species (Meeus, Honnay, Brys, & Jacquemyn, 2012). Yet, higher intra-morph fertilization between L-morphs can make this morph potentially more prone to biparental inbreeding (i.e. due to outcrossing between two L-morphs; Van Rossum & Triest, 2007), which may jeopardize the ability of populations to react to environmental changes.

Although previous evidence has demonstrated deviations of morph ratios from equal frequencies in small plant populations (e.g. Kéry et al., 2003), an understanding of the potential role of recent landscape dynamics and accompanying disruption of plant-pollinator interactions on morph ratios is limited (Barmantlo, Meirmans, Luijten, Triest, & Oostermeijer, 2018). While corresponding findings have attributed deviating morph ratios mainly to stochastic demographic processes, it is not clear whether landscape changes can induce directional deviations of morph frequencies as expected based on the differences in intra-morph fertilization patterns of S- and L-morphs. In this study, we examine morph-ratio patterns in an extensive

number of wild populations of the heterostylous grassland plant *P. veris* across Estonia. To obtain data from landscapes with different history of habitat fragmentation and to cover as wide a geographic scope as possible, we applied a citizen-science approach. In addition to enabling the collection of marked quantities of data, citizen-science projects are increasingly important in raising awareness of conservation issues (Ballard, Phillips, & Robinson, 2018; Turrini, Dörler, Richter, Heigl, & Bonn, 2018), an aspect crucial in an era of vast human-induced environmental change. *Primula veris*, whose abundance has shown a decrease due to recent land-use change (Brys & Jacquemyn, 2009), is thus a suitable species not only for gaining a better understanding of the phenomenon of heterostyly, but enables to increase knowledge of the importance of conserving semi-natural grassland habitats.

Over the last hundred years, the area of the primary habitat of *P. veris* (cowslip), i.e. semi-natural grasslands, has decreased more than 90% in Estonia with potentially detrimental effects on wild plant populations (Laasimer, 1965). We were interested in finding out whether such landscape changes affect the morph balance in the populations of *P. veris*. In particular, we hypothesized that the availability of semi-natural grasslands at the observed sites would yield more equal morph frequencies due to potentially larger population sizes and supporting pollinator communities. In contrast, declines in plant population size and higher proportion of unsuitable habitats replacing former grasslands, such as forests and regions with higher human population density, would lead to higher deviation of morph frequencies from equilibrium conditions. Finally, we hypothesized that in fragmented habitats, morph balance is skewed in favour of L-morphs due to partial intra-morph compatibility among L-morphs.

2 | MATERIALS AND METHODS

2.1 | Study species

Primula veris L. (ssp. *veris*) is a perennial rosette hemicryptophyte typically occurring in calcareous semi-natural grasslands. Being a long-lived, insect-pollinated, habitat-specialist plant, *P. veris* falls into the category of species at a high risk due to factors of global change, such as habitat loss, related pollinator decline and climate change (Estrada et al., 2018). Previous studies have already demonstrated the negative impact of habitat fragmentation on the fitness and neutral genetic diversity of this species (Kéry, Matthies, & Spillmann, 2001; Van Rossum, Campos De Sousa, & Triest, 2004). However, owing to the relatively long life span of the species with an average length of 52.3 years (Ehrlén & Lehtilä, 2002), Lehtilä et al. (2016) observed significant time lags in the demographic patterns of *P. veris* in response to habitat deterioration. Similarly, *P. veris* showed considerable inertia to land-use change after grassland abandonment (Lindborg, Cousins, & Eriksson, 2005).

We considered *P. veris* to be a suitable species for the citizen-science campaign for several reasons. *Primula veris* is a relatively well-known species in Estonia, largely also because of a variety of uses of *P. veris* in folk medicine. Its peak of flowering arrives before most of the other plant species characteristic of semi-natural grasslands, which makes this species easily detectable in the landscape. There are no other morphologically similar species flowering at the same time in Estonia and thus the risk of misidentification is low. The assignment of plants to L- and S-morphs is easy and can be reliably performed by anybody. However, in order to facilitate correct identification of the species as well as S- and L-morphs, the web-platform for uploading data was provided with detailed photos (Figure 1). Furthermore, participants were welcome to upload photos of their own observations, which enabled us to further verify the ability of observers to correctly fulfil the task. The species occurs all across Estonia, but is more widespread in northern and western parts of Estonia because of the higher proportion of suitable habitats (calcareous semi-natural grasslands) in this region.

2.2 | Data collection

The citizen-science campaign '*Eesti otsib nurmenukke*' ('Estonia is looking for cowslips') aimed to engage participants from all over Estonia and a wide range of age groups. Because *P. veris* is flowering before summer holidays, we also invited schools and kindergartens to take part in the campaign. In collaboration with University of Tartu, Estonian Fund for Nature and design studio Fraktal, a user-friendly web-platform (www.nurmenukk.ee, now also available in English on platform www.cowslips.eu) was developed in Estonian and Russian to actively engage the Russian minority in Estonia (the native language of approximately 25%–30% of Estonian inhabitants is Russian). The platform enables easy uploading of all relevant data and GPS coordinates of the study locations with the help of

smartphones. It was also possible to upload data via other devices with internet connection as well as to send data to the campaign organizers as a hard copy. The web-platform included detailed guidelines about carrying out the observations along with explanatory photos (Figure 1; Appendix S1). A more extended guideline in both Estonian and Russian was prepared for biology and kindergarten teachers to foster carrying out the task with a group of children (Appendices S2 and S3). In addition, the web-page included the following short video clips: (a) guidelines about recording morphs, (b) guidelines about making observations without harming the environment, (c) information about the scientific background of the study, (d) information about the folklore and medicinal use of *P. veris* and (e) information about the historic background of heterostyly studies on *P. veris*. All videos were also uploaded in the video-sharing platform YouTube (links provided in Appendix S4).

Each individual observation included the following baseline data: (1) Observation ID, (2) The date of observation, (3) Geographic location (the observer had a choice between automatic localization, writing the location text manually or assigning no location). In addition, the observers were asked the following questions:

- (4) Did you find cowslips? Option: Yes/No.
- (5) Do the cowslips flower? Option: Yes/No.
- (6) Observation of heterostyly. Participants were asked to report the morph type of a hundred individuals wherever it was possible, but were also welcome to submit data on a smaller/higher number of individuals depending on conditions (population size, possibility to spend time for a more extensive sampling effort). Guidelines provided further details for carrying out the observations. In particular, participants were asked to determine the morph of individuals covering the whole area where cowslips were present (i.e. the whole population). After determining the morph of an individual, a participant had to go at least one step further (i.e. about 0.5–1 m) to avoid sampling the same individual. Web-assignment was provided with a clarifying photo and a short description of the particular morphological features and with '+' mark to report a corresponding morph (Figure 1).
- (7) Participants could upload up to five photos depicting the study site.
- (8) People were welcome to add comments, e.g. a more detailed description of the study site, approximate population size and general feedback and suggestions.
- (9) When interested in further feedback, participants could post personal details (the name of the observer, the name of the observing team, e-mail address for feedback correspondence).
- (10) Final step concerned issues about privacy policy and the further use of personal data. It focused on the following key points: (a) the first citizen-science observation of the participant (agree/do not agree), (b) additional data about nature can be sent to the before-mentioned contact information (agree/do not agree) and (c) the further use of privacy data (contact information, names) according to the conditions listed in the privacy policy and for receiving feedback about the outcome of the project (agree/do

not agree). Uploading of heterostyly data did not depend on the agreement/disagreement of the participants with privacy policy. However, we carefully considered these statements during further correspondence with participants and did not include any private data in analyses.

The web-platform and related guidelines were made public in the second half of April in 2019, i.e. the earliest possible date when wild populations of *P. veris* would start flowering in Estonia. Information about the citizen-science project together with guidelines for observations were advertised in a wide range of media platforms: national and local newspapers, Estonian Public Broadcasting TV news and other broadcasts, interviews in different radio programmes and social media (Facebook, Instagram) with live feed to the project web-page. At the end of the campaign, the preliminary results of the project were introduced in outreach articles, in radio broadcasts and in feedback by e-mail to those participants who had agreed to this when filling protocol. In addition, to further acknowledge the contribution of participants, we organized a thanking event, where the most active participants and school teams were rewarded with various prizes.

2.3 | Data analyses

Plant population size has been shown to be a significant determinant of morph deviation from isoplethy (i.e. equal morph ratio; Kéry et al., 2003). Preliminary tests concerning the user-friendliness of the citizen-science platform suggested that the assessment of population size would involve too much uncertainty and potentially decrease the

number of participants. Therefore, the task of evaluating population size was excluded from the protocol. However, in order to take into account the potential effect of population size, we used the number of observed individuals to create a categorical proxy variable with two levels indicating the abundance of plants at the observed location: (a) small populations (<100 individuals); (b) larger populations (≥ 100 observed individuals). The validity of this approach was further verified examining the comments, where many participants reporting the morph of fewer than required 100 individuals mentioned that there were <100 individuals at the observed site.

To examine the potential impact of habitat availability on morph ratios, we calculated the proportion of semi-natural grasslands in the landscapes surrounding those observations, which had been provided with GPS coordinates. The map layer of semi-natural grasslands was obtained using the following sources: (a) the layer of semi-natural grasslands in protected areas (Estonian Environment Agency), (b) the layer of semi-natural grasslands on Natura 2000 network areas (Estonian Environment Agency) and (c) the layer of the database of Estonian Seminatural Community Conservation Association. Because afforestation of landscapes can substantially alter the composition and movement of pollinators with potential consequences for heterostylous plants (Brys & Jacquemyn, 2015), we assessed the area covered by forests and shrubs in the surroundings of the study populations using Estonian Basic Map (1:10,000; Estonian Land Board). The area of semi-natural grasslands suitable for *P. veris* as well as the area of woody elements was assessed in circular buffers with radiuses of 100, 500, 1,000, 2,000 and 5,000 m to take into account both local and large-scale effects of landscape quality (Figure 2).

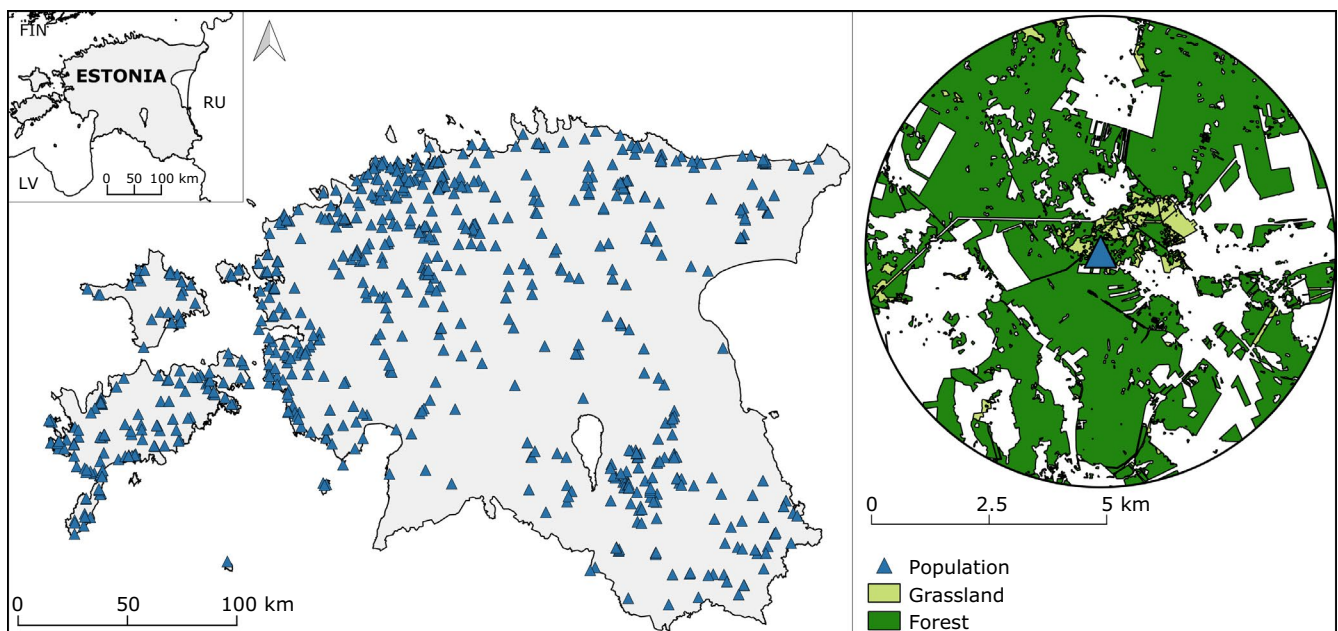


FIGURE 2 Location of the populations of *Primula veris* observed within the frames of the citizen-science project 'Eesti otsib nurmenukke' in Estonia, and an illustration for the buffer approach used for assessing the landscape characteristics (proportion of semi-natural grasslands and woody elements) in the surroundings of the study populations. The figure only includes those observations, which were provided with GPS coordinates

To examine the potential impact of anthropogenic factors (such as infrastructure, other types of higher land-use intensity and/or closeness to urban space potentially affecting pollen flow and habitat quality) on morph deviation, we assessed human population density (people/ha) in 2019 at the study locations using the database of Statistics Estonia (<https://www.stat.ee/>). In addition, we determined the bedrock type of the study locations using the Map of Bedrock (1:400,000; Geological Survey of Estonia 1999, modified by Estonian Land Board in 2019) to take into account the preference of *P. veris* to grow on calcareous soils (Brys & Jacquemyn, 2009). Spatial analyses were done in QGIS version 2.18.14 (QGIS Development Team, 2017).

As we asked participants to consider carrying out the observation across the whole area where *P. veris* was present (i.e. population), we treated the number of reported S- and L-morphs for each observation as a population-based measure. For each such tentative population, we estimated the degree to which the observed proportions of morphs deviate from 1:1 ratio (i.e. if they had equal probability of occurrence). For this, the expected value of S-morphs ($E_{S\text{-morphs}}$) in a population with n individuals was estimated as the expected value of a binomial distribution with a probability $p_{S\text{-morph}} = 0.5$:

$$E_{S\text{-morphs}} = p_{S\text{-morph}} n = 0.5n.$$

We then obtained an estimation of the deviation from expected number of S-morphs by subtracting $E_{S\text{-morphs}}$ from the observed number of S-morphs in each population ($O_{S\text{-morphs}}$). This results in an effect size value ($ES_{S\text{-morphs}} = O_{S\text{-morphs}} - E_{S\text{-morphs}}$). Therefore, positive values of ES indicate that there are more S-morphs than expected in a balanced population, and *vice versa*. However, ES does not inform about how strongly the considered population differs from balance. For example, considering a population with 50 individuals where 30 S-morphs have been observed and a second population with 500 individuals where 300 S-morphs have been observed, both populations have the same proportion of S-morphs. However, the probability that such proportion could be observed in a balanced population is much smaller for the larger population (with 500 individuals sampled) than in the smaller one (50 sampled individuals). For this, following Carmona, Szava-Kovats, and Pärtel (2019), we estimated a standardized effect size (SES) by dividing ES by the standard deviation of the binomial distribution:

$$SES_{S\text{-morphs}} = \frac{ES_{S\text{-morphs}}}{\sqrt{(p_{S\text{-morph}} n) (1 - p_{S\text{-morph}})}} = \frac{ES_{S\text{-morphs}}}{\sqrt{(0.5 n) (1 - 0.5)}}.$$

$SES_{S\text{-morphs}}$ reflects the distance (expressed in standard deviations) between the observed number of S-morphs in a population and the number of S-morphs expected in a balanced population. In the former example, $SES_{S\text{-morphs}}$ in the population with 50 individuals equals 1.41, whereas $SES_{S\text{-morphs}}$ in the population with 500 individuals is 4.47, adequately reflecting the evidence regarding the strength of the deviation from balance in each population. Again, $SES_{S\text{-morphs}}$ will take positive values when S-morphs are more common in the population. Since we

were also interested in analysing the deviation from balance regardless of the directionality of the deviation (i.e. both towards S- or L-morphs), we also estimated the absolute value of $SES_{S\text{-morphs}}$ (SES_{ABS}) for each population.

In addition, to compare the main findings of this study to previous research, we assessed morph ratios using indices applied in previous studies of heterostyly in *P. veris* (i.e. Kéry et al., 2003). In particular, we assessed the proportion of S-morphs ($DEV_{S\text{-morphs}}$; note that Kéry et al. (2003) reported the proportion of L-morphs) and deviation from an even morph frequency (DEV_{ABS} ; sensu Kéry et al., 2003).

We analysed the effect of different proxies for habitat availability and population size both on directional ($SES_{S\text{-morphs}}$ and $DEV_{S\text{-morphs}}$) and absolute deviation (SES_{ABS} and DEV_{ABS}) in populations of *P. veris* using a multi-model inference approach. Preliminary examinations of the different landscape variables (area covered by semi-natural grassland and woody elements around the populations at different radiuses) revealed very high correlations within each of these covers for buffers at similar scales (e.g. forest cover at the 100 m scale is very highly correlated with forest cover at 500 m). However, these correlations were much smaller between the most extreme radiuses considered (100 and 5,000 m; Figure S1). Consequently, to avoid collinearity between predictors, we only used the area covered by semi-natural grasslands and woody elements at 100 m around the populations (as indicators of habitat availability in the local scale) and 5,000 m around the populations (as indicators of habitat availability at the landscape scale). Further, a very large proportion of the examined populations presented a zero area of either semi-natural grasslands (85% populations with an area of 0) or woody elements (56% populations) at the scale of 100 m buffers. Thus, we transformed these variables into binary ones indicating presence (1) or absence (0) of semi-natural grasslands or woody elements in the 100 m buffer surrounding the study populations. We log-transformed human population density to correct for its highly skewed distribution (many sites with low values and a few sites with very high population densities).

Visual examinations of the relationship between the different response variables and the different predictors showed a potential nonlinear effect of human population density, but not of the other variables. Consequently, we fitted linear models where we included the human population density (log-transformed) as a cubic polynomial, and population size (large/small) and the other landscape variables as covariates (grassland and woody element presence at 100 m radius, proportional cover of grasslands and woody elements at 5,000 m radius and bedrock type). We also included all two-way interactions between the human population density and both population size and all landscape variables. We used the MuMIn package (Barton, 2018) to generate subsets of the 'global' model and ranked them using AICc. Then, for each of the four response variables, directional ($SES_{S\text{-morphs}}$ and $DEV_{S\text{-morphs}}$) and absolute (SES_{ABS} and DEV_{ABS}) deviation of morphs, we estimated the relative importance of each predictor as the sum of model weights over all models including each explanatory variable. For each response variable, we

also estimated an averaged model based on Akaike weights, including the subset of models that were within 6 AICc points from the best-ranked model. To explore the effect of important predictors, we created partial regression plots for those predictors that had a significant ($p < 0.05$) effect on the averaged model. Finally, we estimated R^2 from this subset of models by using the Akaike-weighted averaged predicted values (Pistón et al., 2019). We checked spatial autocorrelation of the residuals of the averaged models by means of a Moran's I test. All statistical analyses were performed in R v3.6.0 (R Core Team, 2019).

3 | RESULTS

3.1 | General observations

In total, information about 1,680 observations were uploaded in the web-platform of the citizen-science campaign encompassing the morph type of 225,797 individuals of *P. veris*. Observations covered the whole of Estonia (Figure 2), although more observations were reported from northern and western parts of Estonia, where semi-natural grasslands are more widespread than in eastern and southern Estonia. In addition to semi-natural grasslands and pastures, participants carried out observations in road verges, old quarries, graveyards, verges of health trails and other marginal habitats.

Before further analysis, we carefully reviewed all data to filter out low-quality observations (specific criteria and corresponding number of excluded observations have been listed in Appendix S5), observations without GPS coordinates and observations with <10 observed plant individuals. In total, we included 740 observations into the final analysis about the impact of landscape context on morph ratios. Information about the municipalities, where the observations were made, revealed that fewer than 60 observations originated from urban municipalities. However, most of these were related to smaller towns with a few thousand inhabitants. We are therefore confident that the citizen-science data used in the analyses were not systematically biased towards highly populated regions.

The final dataset included 62,526 S-morphs and 54,002 L-morphs. The mean frequency of S-morphs per population was 53.9% (with standard deviation of $\pm 11.7\%$). Among the observations with ≥ 10 and <100 individuals, which included only one morph (i.e. potential monomorphic populations), 34 observations reported exclusively L-morphs and 19 only S-morphs. However, these observations were excluded from further analyses due to potential uncertainties.

3.2 | Absolute morph deviation

Human population density (cubic polynomial), the presence of semi-natural grasslands in the close surrounding of the populations

(within 100 m) and their interaction were the most important determinants of the standardized absolute deviation from equal morph frequencies (SES_{ABS}) in populations of *P. veris*, and were included in all models with some support according to AICc (Tables 1a and 2). These variables were the only ones with estimates that were statistically different from 0 in the averaged model. Population size (i.e. small and large) also had a relatively high Akaike weight, but its effect was not statistically significant. The rest of the predictors had lower values of importance with bedrock type and the proportion of grasslands within the radius of 5,000 m obtaining the lowest importance (Table 2). The averaged (using Akaike weights) predictions of the selected models explained 17% of the variance in the dataset, and the residuals did not show significant spatial autocorrelation (Moran's $I = -0.003$, $p = 0.819$). SES_{ABS} showed a marked nonlinear pattern with low values in the least densely populated areas and a noticeable increase for population densities higher than one person/ha (Figure 3).

The main determinants of DEV_{ABS} were very similar to those of SES_{ABS} with the main difference that population size played a more important role and was retained in all models selected according to AICc (Table 2). Smaller populations tended to deviate more strongly from even morph frequency than large ones (Figure 4). The averaged model explained 9% of the variance, and there was no significant spatial autocorrelation in its residuals (Moran's $I = 0.007$, $p = 0.363$).

Morph deviation (DEV_{ABS}) and morph deviation accounting for the sample size (SES_{ABS}) were significantly correlated to each other ($r = 0.86$, $p < 0.001$). However, the slopes of the regression lines were different for small and large populations (Figure S2a) demonstrating that the use of deviation indices without accounting for the number of observed individuals may lead to overestimating the magnitude of relative deviation in very small populations.

3.3 | Directional morph deviation

Population size, the presence of semi-natural grasslands at 100 m around the study populations of *P. veris* and human population density and its interactions with population size and presence of grasslands were the variables with the highest importance determining directional morph deviation $SES_{S-morphs}$ according to the model selection procedure (Table 2). None of the other predictors had substantial support with the presence of forests at 100 m around the populations being the next most important variable (importance = 0.52). Similar to the results of the models of absolute morph deviation SES_{ABS} , bedrock type and the proportion of grasslands in the radius of 5,000 m gained the lowest importance in the models predicting $SES_{S-morphs}$ (Table 2). The residuals of the averaged predictions did not show significant spatial autocorrelation (Moran's $I = -0.007$, $p = 0.553$). The proportion of S-morphs was higher in areas with high human population density, but only for populations occurring at semi-natural grasslands (Table 1b; Figure 5). Neither

TABLE 1 Averaged models (estimate, standard deviation and *p*-value) fitted for absolute (a; SES_{ABS} and DEV_{ABS}) and directional (b; $SES_{S-morphs}$ and $DEV_{S-morphs}$) deviation of morph ratios in the study populations of *Primula veris* in Estonia. Variables: grassland (100 m)—presence (1) or absence (0) of grassland areas in a 100 m buffer around the study population; proportion grassland (5,000 m)—proportional grassland area in a 5,000 m buffer (continuous value between 0 and 1); forest (100 m)—presence (1) or absence (0) of woody elements (forests and shrubs) in 100 m buffer; proportion forest (5,000 m)—proportional area occupied by woody elements in a 5,000 m buffer (continuous value between 0 and 1); bedrock (sandstone)—bedrock type at the observation location (takes value 1 for sandstone and 0 for limestone); small population: takes value 1 when the number of individuals sampled in the population is fewer than 100 and 0 otherwise; human density—human population density (continuous variable, number of persons per ha, log-transformed). Statistically significant ($p < 0.05$) parameters have been highlighted in bold font

(a) Absolute deviation from balance (SES_{ABS} and DEV_{ABS})				
	SES_{ABS}		DEV_{ABS}	
	Estimate	<i>p</i>	Estimate	<i>p</i>
Intercept	1.897 ± 0.112	<0.001	0.083 ± 0.005	<0.001
Grassland (100 m)	0.269 ± 0.198	0.174	0.008 ± 0.010	0.422
Proportion grassland (5,000 m)	-0.024 ± 0.024	0.322	-0.001 ± 0.001	0.494
Forest (100 m)	-0.045 ± 0.130	0.733	-0.001 ± 0.007	0.855
Proportion forest (5,000 m)	0.001 ± 0.005	0.868	0	0.981
Bedrock (sandstone)	0.102 ± 0.177	0.565	0 ± 0.009	0.992
Small population	-0.3 ± 0.185	0.106	0.032 ± 0.012	0.005
Log(human density)	4.591 ± 1.947	0.019	0.128 ± 0.109	0.242
Log(human density) ²	3.112 ± 1.933	0.108	0.139 ± 0.107	0.195
Log(human density) ³	0.593 ± 2.022	0.77	0.032 ± 0.107	0.763
Grassland (100 m):small population	0.384 ± 0.486	0.431	0.028 ± 0.024	0.261
Grassland (100 m):log(human density)	29.788 ± 5.530	<0.001	0.744 ± 0.271	0.006
Grassland (100 m):log(human density) ²	22.593 ± 4.222	<0.001	0.426 ± 0.208	0.041
Grassland (100 m):log(human density) ³	13.156 ± 4.101	0.001	0.266 ± 0.201	0.186
Proportion grassland (5,000 m):small population	0.039 ± 0.059	0.512	-0.002 ± 0.003	0.58
Forest (100 m):log(human density)	-6.668 ± 3.492	0.057	-0.321 ± 0.171	0.06
Forest (100 m):log(human density) ²	-1.944 ± 3.452	0.574	-0.097 ± 0.171	0.569
Forest (100 m):log(human density) ³	1.409 ± 3.427	0.682	0.048 ± 0.167	0.773
Forest (100 m):small population	0.137 ± 0.326	0.675	0.026 ± 0.016	0.117
Proportion forest (5,000 m):small population	-0.002 ± 0.012	0.859	0.001 ± 0.001	0.386
Proportion forest (5,000 m):log(human density)	—	—	—	—
Proportion forest (5,000 m):log(human density) ²	—	—	—	—
Proportion forest (5,000 m):log(human density) ³	—	—	—	—
Bedrock (sandstone):small population	-0.224 ± 0.395	0.571	-0.005 ± 0.020	0.78
Grassland (100 m):log(human density)	1.934 ± 4.390	0.66	—	—
Grassland (100 m):log(human density) ²	4.51 ± 4.583	0.326	—	—
Grassland (100 m):log(human density) ³	1.513 ± 4.810	0.754	—	—
Proportion grassland (5,000 m):log(human density)	—	—	0.065 ± 0.049	0.184
Proportion grassland (5,000 m):log(human density) ²	—	—	0.068 ± 0.046	0.135
Proportion grassland (5,000 m):log(human density) ³	—	—	0.028 ± 0.041	0.483
Bedrock (sandstone):log(human density)	3.462 ± 5.301	0.514	0.282 ± 0.26	0.277
Bedrock (sandstone):log(human density) ²	3.189 ± 5.003	0.525	0.172 ± 0.243	0.481
Bedrock (sandstone):log(human density) ³	7.447 ± 5.370	0.166	0.555 ± 0.262	0.034
Small population:log(human density)	—	—	0.389 ± 0.218	0.074
Small population:log(human density) ²	—	—	0.379 ± 0.227	0.095
Small population:log(human density) ³	—	—	-0.03 ± 0.234	0.897

(Continues)

TABLE 1 (Continued)

(b) Directional deviation from balance ($SES_{S-morphs}$ and $DEV_{S-morphs}$)				
	$SES_{S-morphs}$		$DEV_{S-morphs}$	
	Estimate	<i>p</i>	Estimate	<i>p</i>
Intercept	0.872 ± 0.178	<0.001	0.539 ± 0.009	<0.001
Grassland (100 m)	1.014 ± 0.276	<0.001	0.037 ± 0.014	0.009
Proportion grassland (5,000 m)	-0.021 ± 0.034	0.544	-0.001 ± 0.002	0.768
Forest (100 m)	0.021 ± 0.202	0.917	-0.003 ± 0.010	0.784
Proportion forest (5,000 m)	-0.005 ± 0.008	0.502	—	—
Bedrock (sandstone)	-0.21 ± 0.261	0.423	-0.009 ± 0.013	0.484
Small population	-0.747 ± 0.393	0.057	-0.047 ± 0.018	0.008
Log(human density)	3.644 ± 3.174	0.252	0.135 ± 0.173	0.436
Log(human density) ²	2.352 ± 3.122	0.452	0.093 ± 0.165	0.576
Log(human density) ³	-2.35 ± 3.337	0.482	-0.164 ± 0.176	0.35
Grassland (100 m):small population	0.687 ± 0.683	0.316	0.076 ± 0.034	0.024
Grassland (100 m):log(human density)	33.315 ± 7.684	<0.001	0.905 ± 0.372	0.015
Grassland (100 m):log(human density) ²	27.885 ± 5.893	<0.001	0.725 ± 0.284	0.011
Grassland (100 m):log(human density) ³	13.09 ± 5.744	0.023	0.249 ± 0.277	0.37
Proportion grassland (5,000 m):small population	-0.015 ± 0.088	0.864	-0.005 ± 0.004	0.293
Forest (100 m):log(human density)	-6.277 ± 4.916	0.202	-0.391 ± 0.237	0.101
Forest (100 m):log(human density) ²	4.369 ± 4.885	0.372	0.234 ± 0.233	0.318
Forest (100 m):log(human density) ³	5.608 ± 4.798	0.243	0.291 ± 0.231	0.209
Forest (100 m):small population	0.819 ± 0.469	0.081	0.072 ± 0.023	0.002
Proportion forest (5,000 m):small population	0.024 ± 0.018	0.188	0.001 ± 0.001	0.443
Proportion forest (5,000 m):log(human density)	-0.279 ± 0.245	0.255	-0.015 ± 0.012	0.214
Proportion forest (5,000 m):log(human density) ²	0.006 ± 0.220	0.977	0 ± 0.0110	0.966
Proportion forest (5,000 m):log(human density) ³	0.27 ± 0.200	0.177	0.012 ± 0.010	0.212
Bedrock (sandstone):small population	0.676 ± 0.559	0.228	0.038 ± 0.027	0.169
Grassland (100 m):log(human density)	—	—	—	—
Grassland (100 m):log(human density) ²	—	—	—	—
Grassland (100 m):log(human density) ³	—	—	—	—
Proportion grassland (5,000 m):log(human density)	—	—	0.067 ± 0.067	0.316
Proportion grassland (5,000 m):log(human density) ²	—	—	0.071 ± 0.063	0.259
Proportion grassland (5,000 m):log(human density) ³	—	—	-0.012 ± 0.056	0.835
Bedrock (sandstone):log(human density)	—	—	—	—
Bedrock (sandstone):log(human density) ²	—	—	—	—
Bedrock (sandstone):log(human density) ³	—	—	—	—
Small population:log(human density)	0.854 ± 6.242	0.891	0.21 ± 0.303	0.489
Small population:log(human density) ²	-10.3 ± 6.483	0.115	-1.345 ± 0.315	<0.001
Small population:log(human density) ³	-4.221 ± 6.663	0.527	-0.405 ± 0.325	0.213

population size nor its interaction with human population density had significant effects on the proportion of *S-morphs*. The averaged predictions explained 14% of total variance.

The main determinants of $DEV_{S-morphs}$ were the same as for $SES_{S-morphs}$, with the only addition of the presence of forests at 100 m around the study populations of *P. veris* (Table 2), which was included in all selected models mostly because of its

interactions with other variables (Table 1b). The averaged model explained 10% of the total variance, and there was no significant spatial autocorrelation in the residuals (Moran's $I = -0.006$, $p = 0.628$).

Directional morph deviation ($DEV_{S-morphs}$) and morph deviation accounting for the number of observed individuals ($SES_{S-morphs}$) were significantly correlated ($r = 0.922$, $p < 0.001$; Figure S2b).

TABLE 2 Relative importance of the variables included in each model about the effect of human population density, population size (small/large) and landscape variables on the deviation of morph frequencies from isoplethy in populations of *Primula veris* in Estonia. For each explanatory variable we report the sum of Akaike weights of models including that variable, among the models within 6 AICc points of the best model, as well as the number of models that included it (in parenthesis)

Predictor	SES _{ABS}	DEV _{ABS}	SES _{S-morphs}	DEV _{S-morphs}
Human density	1 (66)	1 (152)	1 (105)	1 (51)
Grassland (100 m)	1 (66)	1 (152)	1 (105)	1 (51)
Grassland (100 m):human density	1 (66)	1 (152)	1 (105)	1 (51)
Small population	0.78 (53)	1 (152)	1 (105)	1 (51)
Proportion grassland (5,000 m)	0.36 (25)	0.34 (68)	0.28 (40)	0.33 (24)
Forest (100 m)	0.29 (30)	0.41 (77)	0.52 (63)	1 (51)
Bedrock	0.29 (26)	0.31 (66)	0.35 (47)	0.38 (26)
Proportion forest (5,000 m)	0.24 (23)	0.25 (51)	0.37 (51)	0.38 (26)
Grassland (100 m):small population	0.21 (16)	0.38 (65)	0.33 (38)	0.9 (42)
Proportion grassland (5,000 m):small population	0.06 (5)	0.07 (19)	0.05 (9)	0.09 (7)
Bedrock:small population	0.05 (5)	0.04 (11)	0.14 (20)	0.17 (12)
Forest (100 m):small population	0.04 (6)	0.23 (42)	0.35 (42)	0 (0)
Forest (100 m):human density	0.04 (6)	0.06 (14)	0.06 (11)	0.39 (23)
Proportion forest (5,000 m):small population	0.03 (5)	0.07 (15)	0.15 (21)	0.10 (9)
Small population:human density	<0.001 (1)	0.53 (79)	0.97 (98)	1 (51)
Bedrock:human density	<0.001 (1)	0.1 (22)	0 (0)	0 (0)
Proportion forest (5,000 m):human density	0 (0)	0 (0)	0.02 (5)	0.02 (2)
Proportion grassland (5,000 m):human density	0 (0)	<0.01 (3)	0 (0)	<0.01 (1)

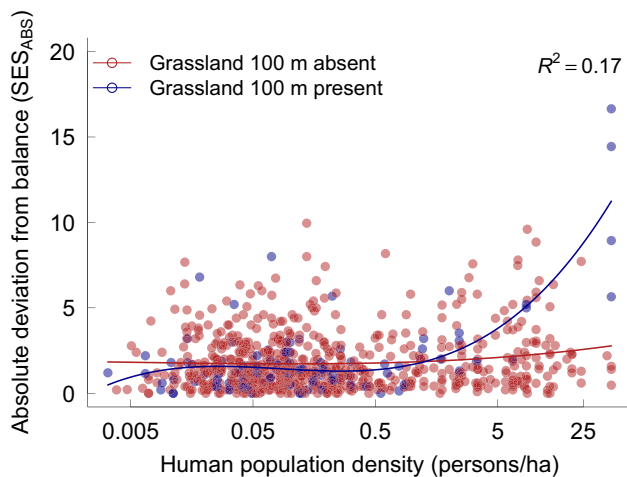


FIGURE 3 The effect of human population density and its interaction with the presence of semi-natural grasslands at the study population or in the closest proximity (within the buffer with a radius of 100 m) on absolute morph frequencies in the populations of *Primula veris* in Estonia recorded in the frames of the citizen-science campaign. Summary of the fitted models is presented in Table 1a. R^2 represents the explained variance in the dataset according to the averaged (using Akaike weights) predictions of the selected models

4 | DISCUSSION

In this study, we examined the effect of landscape context on the morph ratios of the distylous grassland plant *P. veris* in Estonia, where

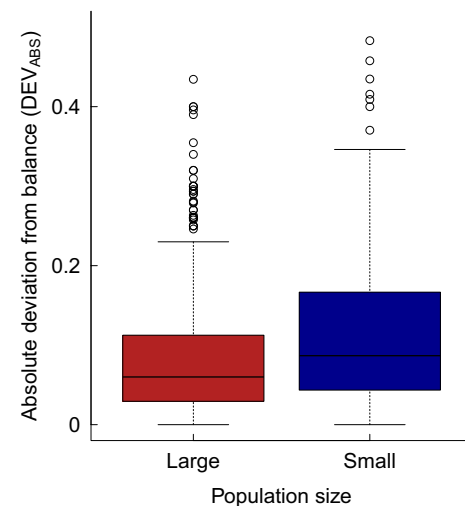


FIGURE 4 The effect of plant population size on absolute morph frequencies (DEV_{ABS}) in the populations of *Primula veris* in Estonia recorded in the frames of the citizen-science campaign. Summary of the fitted models is presented in Table 1a

the area and connectivity of semi-natural grasslands has substantially decreased over the last century. A successful citizen-science campaign enabled us to obtain data from nearly 1,700 populations all across Estonia. S-morphs were more prevalent in the dataset compared to L-morphs. However, data from 740 observations, which met the criteria for further statistical analyses, revealed that landscape context had a significant effect on morph frequencies. In particular, morph

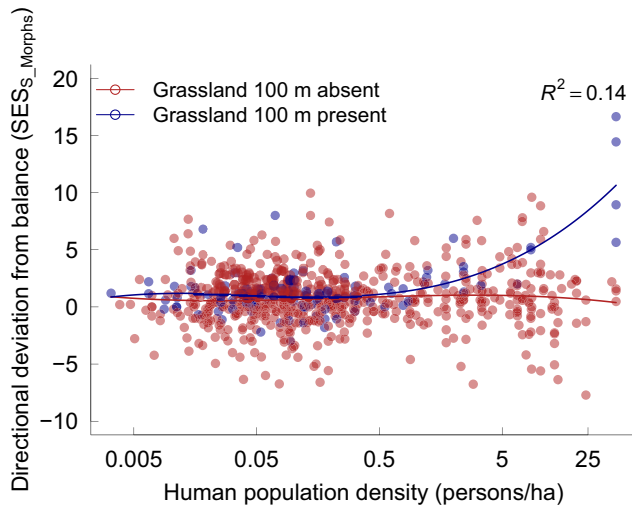


FIGURE 5 The effect of human population density and its interaction with the presence of semi-natural grasslands at the study population (within the buffer with a radius of 100 m) on directional deviation of morph balance ($SES_{S-morphs}$) in Estonia recorded within the citizen-science campaign. Higher values indicate to higher proportion of S-morphs. Summary of the fitted models is presented in Table 1b. R^2 represents the explained variance in the dataset according to the averaged (using Akaike weights) predictions of the selected models

frequencies of *P. veris* were more likely to deviate in semi-natural grasslands located in regions with higher human population density (Figure 3). Furthermore, the deviation in such grasslands was more often in favour of S-morphs (Figure 5). We also found that smaller populations were more prone to overall morph deviations (Figure 4).

4.1 | Absolute morph deviation

We found that an increase in human population density in the surroundings of the observed populations caused a significant deviation of morphs from isopleth (Figure 3). In particular, plant populations in semi-natural grasslands located in regions with human population density higher than one person per hectare tended to have unbalanced morph frequencies. The average human population density for the whole of Estonia is relatively low with 0.31 persons/ha. Increasing human population density may indicate various processes in relation to anthropogenic pressure, such as urbanization and infrastructural development, but also higher rural human population in areas with high-intensity agriculture leading to the loss and fragmentation as well as decreased quality of habitats. Among other major shifts in landscape change in Estonia, such as the overgrowth of semi-natural grasslands with trees and bushes following the lack of management (Pärtel, Mändla, & Zobel, 1999), recent history has witnessed substantial urbanization and related urban sprawl in the surroundings of the larger settlements in Estonia. Suburban development has mainly occurred on former grasslands encompassing the suitable habitats of the study species *P. veris* (Roose, Kull, Gauk, & Tali, 2013).

Pressure from urban sprawl generally imposes a strong negative environmental impact, including the loss of natural and semi-natural habitats, and lower quality of the remaining habitats (Hansen et al., 2005; Sushinsky, Rhodes, Possingham, Gill, & Fuller, 2013) leading to the loss of typical grassland species (Saar, Takkis, Pärtel, & Helm, 2012). *Primula veris* is a species characteristic to semi-natural grasslands, which in Estonia require continuous and regular moderate human disturbance through mowing and grazing for persistence. Indeed, moderate historic human population densities (most likely reflecting patterns of historic grassland management) was shown to have a positive effect on biodiversity (Helm et al., 2009; Pärtel, Helm, Reitalu, Liira, & Zobel, 2007). However, in the same study system, contemporary human population densities had an adverse effect on the genetic diversity of a grassland-specialist plant (Helm et al., 2009). Similarly, our study indicates that contemporary human population density can skew patterns of morph deviations of *P. veris* in semi-natural grasslands. Demographic instability as a result of abrupt plant population collapses (Kiviniemi, 2008) and related population bottlenecks bring along stochastic mortality of individuals, which can easily lead to deviated morph frequencies in populations having recently experienced loss in size. This has been confirmed by significant deviations of morph frequencies in small populations of *P. veris* (Brys, Jacquemyn, Endels, Hermy, & De Blust, 2003; Kéry et al., 2003), but also in other heterostylous species, such as *P. vulgaris* (Endels et al., 2002) and *Pulmonaria officinalis* (Brys, Jacquemyn, & Beeckman, 2008).

Models with different indices of deviation (SES_{ABS} vs. DEV_{ABS}) led to relatively comparable results. However, overall deviation of morphs from balance (DEV_{ABS} ; index sensu Kéry et al., 2003) depended also on population size: smaller populations (with fewer than 100 observed individuals) were characterized by higher deviation (Figure 4). Interestingly, this finding was not confirmed for SES_{ABS} , a measure standardized according to the number of observed individuals. Although frequencies of different morphs are much more prone to random fluctuation in smaller populations (Kéry et al., 2003), it is also probable that proportional deviation without standardization in relation to the number of observed individuals may not provide unbiased understanding of the mechanisms driving patterns of morph ratios. To take into account such biases, we mainly focused on standardized estimates of deviation (SES_{ABS} and $SES_{S-morphs}$).

4.2 | Directional morph deviation

Surprisingly, S-morphs were more abundant in the overall dataset on average (nearly 54% of analysed individuals were S-morphs), and had a higher proportion per population as well. Furthermore, S-morphs were more prevalent in semi-natural grasslands or locations where semi-natural grasslands were nearby (i.e. in the buffer area of 100 m) in regions with high human population density (Figure 5). Following the Mendelian inheritance patterns, crosses between compatible morphs should yield an equal number of S- and L-morphs, which

is why the overall dominance of S-morphs is thought-provoking. Among previous research, only Lees (1971) recorded the dominance of S-morphs over L-morphs in *P. veris* and assigned this phenomenon to the potential advantage of heterozygous S-morphs in certain microclimates. Similarly, a sample of 522 individuals of *P. veris* collected by Charles Darwin exhibited an excess of 281 S-morphs over 241 L-morphs (Darwin, 1862). However, the number of sampled populations in those studies was too small to draw broader conclusions. In contrast, Kéry et al. (2003) who recorded the morph frequencies in 76 populations of *P. veris* in Switzerland, found no differences in the overall frequency of L- and S-morphs nor differences between the mean values per population.

One of the reasons for the overall prevalence of S-morphs could be the relatively higher fitness and survival of S-morphs owing to a stricter incompatibility mechanism among S-morphs and hence potentially higher genetic diversity. L-morphs, where a certain degree of within-morph compatibility has been observed (Wedderburn & Richards, 1990), may therefore exhibit higher (biparental) inbreeding, which can lead to lower survival of L-morphs. Indeed, Van Rossum and Triest (2007) reported higher kinship coefficients in L-morphs compared to S-morphs in fragmented populations of *P. veris* lending support to our hypothesis about the relatively higher inbreeding in L-morphs. Preliminary genetic analysis of heterostyly data from fragmented semi-natural grasslands in western Estonia revealed moderate inbreeding in L-morphs, but surprisingly more so in populations dominated by S-morphs (Kaldra, 2020). It is, however, also possible that in such populations, high inbreeding in L-morphs has already led to the lower number of long-styled individuals compared to S-morphs. In the peripheral populations of *P. merrilliana*, Shao et al. (2019) observed intra-morph compatibility as well as self-compatibility not only in L-morphs, but also at similar frequencies in S-morphs. Furthermore, self- and intra-morph fertilization in S-morphs yielded a higher seed set than self- and intra-morph crosses in L-morphs of this species, which would help to buffer the increase of L-morphs as a result of intra-morph crosses (yielding to pins only). Thus, the variation of the patterns of intra-morph compatibility and self-compatibility in heterostylous plants may be higher than previously assumed and can also depend on the location of the population in relation to species' distributional range.

The drastic loss of semi-natural grassland habitats has disrupted vital plant-pollinator interactions necessary for successful reproduction (Valiente-Banuet et al., 2015). For outcrossing plant species depending on pollinator activity, the chronic lack of pollinators in combination with decreasing size of populations may favour phenotypes that are capable of partial self-compatibility or, in heterostylous species, partial intra-morph compatibility. Lower probability of legitimate pollen transfer can eventually lead to the loss of heterostyly (Barmantlo et al., 2018; Shao et al., 2019; Yuan et al., 2017). In our dataset, 53 observations (among those provided with GPS coordinates and with ≥ 10 individuals) included only one morph. These observations included fewer than 100 observed flowering individuals indicating small population size. Interestingly, 34 of those reported only L-morphs, while 19 observations comprised of only S-morphs,

although the overall data included more S-morphs. Nearly all monomorphic observations originated from locations with no semi-natural grasslands nearby. Although monomorphism of *P. veris* in those locations needs to be further validated on site, these findings seem to suggest that the loss of suitable habitats may potentially indeed lead to drastic deviations of morphs with potential effects on fitness (Kéry et al., 2003) and evolutionary consequences, such as the loss of distyly. The dominance of long-styled monomorphism among our observations supports the hypothesis that partial intra-morph compatibility may to a certain extent be advantageous in alternative habitats of landscapes with no semi-natural grasslands, such as road verges, field boundaries and fallow land. The latter was also confirmed by the moderate prevalence of L-morphs in small plant populations in landscapes with higher human impact (Table 1b). However, what the long-term consequences of extreme deviations of morph frequencies are and whether some of these populations are indeed evolving towards the breakdown of heterostyly, needs further verification.

4.3 | Engaging people in citizen science

Citizen-science approaches for collecting environmental data have gained growing interest over the last decade (Silvertown, 2009; Turrini et al., 2018). Wider engagement of people in ecological research has multi-faceted benefits, from enhancing public knowledge in biodiversity and conservation issues to collecting scientific data at unprecedented spatial and temporal scales, which is often unattainable with traditional scientific methods (Ballard et al., 2018; Cooper, Dickinson, Phillips, & Bonney, 2007). Although we did not specifically assess the impact of our citizen-science approach on raising awareness of ecological knowledge and conservational issues, it was apparent from numerous comments uploaded by the participants that the impact was far beyond increasing knowledge just on heterostyly. In general, based on the comments, aspects covered by the citizen-science campaign apart from the main task of the campaign can be divided broadly into the following sub-topics: landscape change, ecosystem services, botanical observations, interactions of observed *P. veris* individuals with other organism groups (herbivores and pollinators), habitats, where the observed populations were found (semi-natural grasslands, but quite often also in alternative habitats, such as road verges, quarries, former dump sites, airfield, graveyards etc) and environmental education. Thus, somewhat surprisingly, despite the rather specific task (i.e. determination of morph types in *P. veris*), people often approached the assignment more holistically. Comprehensive background information (e.g. explaining the wider implications of landscape change for biodiversity) provided in the web-platform as well as in media may have been among the potential reasons why people actually noticed more than asked to report in the data form. Hence, such citizen-science projects may have wider implications than just enhanced knowledge on the particular subject.

Nearly 250 participating teams originated from schools or kindergartens. Positive feedback from teachers provided in the comments

section of the protocol revealed that such participatory approaches offer very valuable educational experiences complementing the compulsory curriculum with both theoretical introduction into the subject as well as with related hands-on activities, which help to understand the topic. Furthermore, several teachers showed interest to participate in any follow-up projects on monitoring of heterostyly. Understanding of the temporal dynamics of morph ratios is very scarce (Endels et al., 2002). Thus, both from educational angle and from the scientific point of view, continuation of the citizen-science project on heterostyly and expanding its geographic scope is surely worthy of further consideration.

4.4 | Implications for nature conservation

A number of *P. veris* populations observed in the current citizen-science campaign were located in regions exposed to relatively high human pressure, such as the county surrounding the largest town in Estonia, Tallinn. Urban sprawl and related human disturbances pose great challenges for maintaining biodiversity (Hansen et al., 2005). However, different types of semi-natural elements related to infrastructural and urban habitats also represent opportunities for supporting diverse wild plant and pollinator communities in case such elements are managed in a biodiversity-friendly way (Phillips, Gaston, Bullock, & Osborne, 2019; Watson, Carignan-Guillemette, Turcotte, Maire, & Proulx, 2019).

Morph deviation in the populations of *P. veris* appears to be to certain extent determined by stochastic demographic processes in fragmented habitats characterized by lower abundance of plant individuals and higher human population density. Furthermore, it is possible that the higher abundance of monomorphic populations with L-morphs reported in habitats with no semi-natural grasslands in the surroundings and the prevalence of L-morphs in small populations in landscapes with high human impact refers to the role of deterministic processes, such as the partial capability of L-morphs intra-morph fertilization. On the other hand, S-morphs were prevailing in most populations, particularly in semi-natural grasslands of landscapes experiencing higher human impact. Such patterns may affect the future viability of heterostylous species in various ways. Previous research suggests that strong deviations in morph frequencies may further contribute to the loss of genetic variation (Meeus et al., 2012) and thus exacerbate the extinction vortex of heterostylous plants in fragmented habitats. Similarly, recent findings in western Estonia show that populations of *P. veris* with unbalanced morph frequencies exhibit lower genetic diversity (Kaldra, 2020). Decreased genetic diversity in populations with deviated morph frequencies also make them more vulnerable to climate change and other environmental perturbations (Leigh, Hendry, Vázquez-Domínguez, & Friesen, 2019).

Novel selection pressures, such as the scarcity of suitable mates and lack of pollinating insects, may eventually lead to both stochastic and directional deviations of morphs and, ultimately, to evolutionary changes in reproductive traits (Jacquemyn et al., 2012). In

addition to the revealed effects of landscape context on morph deviations in *P. veris*, this study yielded a number of observations with only a single morph present, particularly in small populations. These findings need to be examined further in order to clarify what the mechanisms for the unbalanced morph frequencies are and whether the development of homostyly as a result of recent landscape change is more common than observed until now.

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AUTHORS' CONTRIBUTIONS

T.A., M.P., I.H. and S.L. conceived the project idea; M.K., A.H., K.H., M.K., T.O. and S.L. helped to set up the citizen-science campaign and promote the campaign among different target groups; C.P.C., S.T., M.K., I.R. and T.A. contributed to data management and statistical analyses; T.A. wrote the first draft and all authors provided input and approved the final version of the manuscript.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data about the morph frequencies of *Primula veris* in Estonia collected in the frames of the citizen-science campaign have been uploaded in PlutoF Data Management and Publishing Platform (<https://plutof.ut.ee/#/study/view/81083>).

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REFERENCES

- Aavik, T., & Helm, A. (2018). Restoration of plant species and genetic diversity depends on landscape-scale dispersal. *Restoration Ecology*, 26, S92–S102.
- Aavik, T., Holderegger, R., Edwards, P. J., & Billeter, R. (2013). Patterns of contemporary gene flow suggest low functional connectivity of grasslands in a fragmented agricultural landscape. *Journal of Applied Ecology*, 50, 395–403.
- Aavik, T., Jõgar, Ü., Liira, J., Tulva, I. & Zobel, M. (2008). Plant diversity in a calcareous wooded meadow – The significance of management continuity. *Journal of Vegetation Science*, 19, 475–484.
- Aguilar, R., Ashworth, L., Galetto, L., & Aizen, M. A. (2006). Plant reproductive susceptibility to habitat fragmentation: Review and synthesis through a meta-analysis. *Ecology Letters*, 9, 968–980.
- Ash, J. D., Givnish, T. J., & Waller, D. M. (2017). Tracking lags in historical plant species' shifts in relation to regional climate change. *Global Change Biology*, 23, 1305–1315.
- Auffret, A. G., Kimberley, A., Plue, J., & Waldén, E. (2018). Super-regional land-use change and effects on the grassland specialist flora. *Nature Communications*, 9, 3464.
- Ballard, H. L., Phillips, T. B., & Robinson, L. (2018). Conservation outcomes of citizen science. In S. Hecker, M. Haklay, A. Bowser, Z. Makuch, J. Vogel, & A. Bonn (Eds.), *Citizen science: Innovation in open science, society and policy* (pp. 254–268). London, UK: UCL Press.
- Barmantlo, S. H., Meirns, P. G., Luijten, S. H., Triest, L., & Oostermeijer, J. G. B. (2018). Outbreeding depression and breeding system evolution in small, remnant populations of *Primula vulgaris*: Consequences for genetic rescue. *Conservation Genetics*, 19, 545–554. <https://doi.org/10.1007/s10592-017-1031-x>
- Barrett, S. C. H., & Shore, J. S. (2008). New insights on heterostyly: Comparative biology, ecology and genetics. In V. E. Franklin-Tong (Ed.), *Self-incompatibility in flowering plants: Evolution, diversity, and mechanisms* (pp. 3–32). Berlin, Heidelberg, Germany: Springer Berlin Heidelberg.
- Barton, K. (2018). *MuMIn: Multi-model inference*. R-package version 1.40.4.
- Bastian, O. (2013). The role of biodiversity in supporting ecosystem services in Natura 2000 sites. *Ecological Indicators*, 24, 12–22.
- Biesmeijer, J. C., Roberts, S. P. M., Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T., ... Kunin, W. E. (2006). Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science*, 313, 351–354.
- Bodbyl Roels, S. A., & Kelly, J. K. (2011). Rapid evolution caused by pollinator loss in *Mimulus guttatus*. *Evolution*, 65, 2541–2552.
- Brys, R., & Jacquemyn, H. (2009). Biological flora of the British Isles: *Primula veris* L. *Journal of Ecology*, 97, 581–600.
- Brys, R., & Jacquemyn, H. (2015). Disruption of the distylous syndrome in *Primula veris*. *Annals of Botany*, 115, 27–39.
- Brys, R., & Jacquemyn, H. (2020). The impact of individual inaccuracy of reciprocal herkogamy on legitimate pollen deposition and seed set in a distylous self-incompatible herb. *Journal of Ecology*, 108, 81–93. <https://doi.org/10.1111/1365-2745.13258>
- Brys, R., Jacquemyn, H., & Beeckman, T. (2008). Morph-ratio variation, population size and female reproductive success in distylous *Pulmonaria officinalis* (Boraginaceae). *Journal of Evolutionary Biology*, 21, 1281–1289.
- Brys, R., Jacquemyn, H., Endels, P., Herm, M., & De Blust, G. (2003). The relationship between reproductive success and demographic structure in remnant populations of *Primula veris*. *Acta Oecologica*, 24, 247–253.
- Carmona, C. P., Szava-Kovats, R., & Pärtel, M. (2019). Estimating probabilistic dark diversity based on the hypergeometric distribution. *bioRxiv*, 636753.
- Clough, Y., Ekroos, J., Báldi, A., Batáry, P., Bommarco, R., Gross, N., ... Smith, H. G. (2014). Density of insect-pollinated grassland plants decreases with increasing surrounding land-use intensity. *Ecology Letters*, 17, 1168–1177.
- Cooper, C. B., Dickinson, J., Phillips, T., & Bonney, R. (2007). Citizen science as a tool for conservation in residential ecosystems. *Ecology and Society*, 12, 11.
- Cousins, S. A. O., Auffret, A. G., Lindgren, J., & Tränk, L. (2015). Regional-scale land-cover change during the 20th century and its consequences for biodiversity. *Ambio*, 44, 17–27.
- Darwin, C. (1862). On the two forms, or dimorphic condition, in the species of *Primula*, and on their remarkable sexual relations. *Journal of the Proceedings of the Linnean Society of London. Botany*, 6, 77–96.
- Ehrlén, J., & Lehtilä, K. (2002). How perennial are perennial plants? *Oikos*, 98, 308–322.
- Endels, P., Jacquemyn, H., Brys, R., & Herm, M. (2002). Changes in pin-thrum ratios in populations of the heterostyle *Primula vulgaris* Huds.: Does imbalance affect population persistence? *Flora – Morphology, Distribution, Functional Ecology of Plants*, 197, 326–331.
- Estrada, A., Morales-Castilla, I., Meireles, C., Caplat, P., & Early, R. (2018). Equipped to cope with climate change: Traits associated with range filling across European taxa. *Ecography*, 41, 770–781. <https://doi.org/10.1111/ecog.02968>
- Habel, J. C., Dengler, J., Janišová, M., Török, P., Wellstein, C., & Wiek, M. (2013). European grassland ecosystems: Threatened hotspots of biodiversity. *Biodiversity and Conservation*, 22, 2131–2138.
- Hansen, A. J., Knight, R. L., Marzluff, J. M., Powell, S., Brown, K., Gude, P. H., & Jones, K. (2005). Effects of exurban development on biodiversity: Patterns, mechanisms, and research needs. *Ecological Applications*, 15, 1893–1905.
- Helm, A., Oja, T., Saar, L., Takkis, K., Talve, T., & Pärtel, M. (2009). Human influence lowers plant genetic diversity in communities with extinction debt. *Journal of Ecology*, 97, 1329–1336.
- Honnay, O., & Jacquemyn, H. (2007). Susceptibility of common and rare plant species to the genetic consequences of habitat fragmentation. *Conservation Biology*, 21, 823–831.
- Hoofman, D. A. P., & Bullock, J. M. (2012). Mapping to inform conservation: A case study of changes in semi-natural habitats and their connectivity over 70 years. *Biological Conservation*, 145, 30–38.
- Huu, C. N., Kappel, C., Keller, B., Sicard, A., Takebayashi, Y., Breuninger, H., ... Lenhard, M. (2016). Presence versus absence of CYP734A50 underlies the style-length dimorphism in primroses. *eLife*, 5, e17956.
- Jacquemyn, H., Brys, R., & Herm, M. (2002). Patch occupancy, population size and reproductive success of a forest herb (*Primula elatior*) in a fragmented landscape. *Oecologia*, 130, 617–625.
- Jacquemyn, H., De Meester, L., Jongejans, E., & Honnay, O. (2012). Evolutionary changes in plant reproductive traits following habitat fragmentation and their consequences for population fitness. *Journal of Ecology*, 100, 76–87.
- Jakobsson, A., & Ågren, J. (2014). Distance to semi-natural grassland influences seed production of insect-pollinated herbs. *Oecologia*, 175, 199–208.
- Kaldra, M. (2020). *The role of heterostyly for maintaining genetic diversity in Primula veris* (Master thesis). University of Tartu, Tartu.
- Kéry, M., Matthies, D., & Schmid, B. (2003). Demographic stochasticity in population fragments of the declining distylous perennial *Primula veris* (Primulaceae). *Basic and Applied Ecology*, 4, 197–206.
- Kéry, M., Matthies, D., & Spillmann, H.-H. (2001). Reduced fecundity and offspring performance in small populations of the declining grassland plants *Primula veris* and *Gentiana lutea*. *Journal of Ecology*, 88, 17–30. <https://doi.org/10.1046/j.1365-2745.2000.00422.x>
- Kiviniemi, K. (2008). Effects of fragment size and isolation on the occurrence of four short-lived plants in semi-natural grasslands. *Acta Oecologica*, 33, 56–65.
- Kohler, F., Verhulst, J., van Klink, R., & Kleijn, D. (2008). At what spatial scale do high-quality habitats enhance the diversity of forbs and pollinators in intensively farmed landscapes? *Journal of Applied Ecology*, 45, 753–762.

- Laasimer, L. (1965). *Eesti NSV Taimkate*. Tallinn, Estonia: Eesti NSV Teaduste Akadeemia Zoologia ja Botaanika Instituut. Kirjastus 'Valgus'.
- Lees, D. R. (1971). Frequencies of pin and thrum plants in a wild population of the Cowslip, *Primula veris* L. *Watsonia*, 8, 289–291.
- Lehtilä, K., Dahlgren, J. P., Garcia, M. B., Leimu, R., Syrjänen, K., & Ehrlén, J. (2016). Forest succession and population viability of grassland plants: Long repayment of extinction debt in *Primula veris*. *Oecologia*, 181, 125–135. <https://doi.org/10.1007/s00442-016-3569-6>
- Leigh, D. M., Hendry, A. P., Vázquez-Domínguez, E., & Friesen, V. L. (2019). Estimated six per cent loss of genetic variation in wild populations since the industrial revolution. *Evolutionary Applications*, 12, 1505–1512.
- Li, J., Webster, M., Furuya, M., & Gilmartin, P. M. (2007). Identification and characterization of pin and thrum alleles of two genes that co-segregate with the *Primula* S locus. *The Plant Journal*, 51, 18–31.
- Lindborg, R., Cousins, S. A. O., & Eriksson, O. (2005). Plant species response to land use change – *Campanula rotundifolia*, *Primula veris* and *Rhinanthus minor*. *Ecography*, 28, 29–36.
- Lindgren, J., Lindborg, R., & Cousins, S. A. O. (2018). Local conditions in small habitats and surrounding landscape are important for pollination services, biological pest control and seed predation. *Agriculture, Ecosystems & Environment*, 251, 107–113.
- Meeus, S., Honnay, O., Brys, R., & Jacquemyn, H. (2012). Biased morph ratios and skewed mating success contribute to loss of genetic diversity in the distylous *Pulmonaria officinalis*. *Annals of Botany*, 109, 227–235.
- Nowak, M., Russo, G., Schlapbach, R., Huu, C., Lenhard, M., & Conti, E. (2015). The draft genome of *Primula veris* yields insights into the molecular basis of heterostyly. *Genome Biology*, 16, 12.
- Öckinger, E., & Smith, H. G. (2007). Semi-natural grasslands as population sources for pollinating insects in agricultural landscapes. *Journal of Applied Ecology*, 44, 50–59.
- Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, 120, 321–326.
- Ornduff, R. (1980). Pollen flow in *Primula veris* (Primulaceae). *Plant Systematics and Evolution*, 135, 89–93.
- Pärtel, M., Helm, A., Reitalu, T., Liira, J., & Zobel, M. (2007). Grassland diversity related to the Late Iron Age human population density. *Journal of Ecology*, 95, 574–582.
- Pärtel, M., Mändla, R., & Zobel, M. (1999). Landscape history of a calcareous (alvar) grassland in Hanila, western Estonia, during the last three hundred years. *Landscape Ecology*, 14, 187–196.
- Phillips, B. B., Gaston, K. J., Bullock, J. M., & Osborne, J. L. (2019). Road verges support pollinators in agricultural landscapes, but are diminished by heavy traffic and summer cutting. *Journal of Applied Ecology*, 56, 2316–2327.
- Pico, F. X., & van Groenendael, J. (2007). Large-scale plant conservation in European semi-natural grasslands: A population genetic perspective. *Diversity and Distributions*, 13, 920–926.
- Pistón, N., de Bello, F., Dias, A. T. C., Götzenberger, L., Rosado, B. H. P., de Mattos, E. A., ... Carmona, C. P. (2019). Multidimensional ecological analyses demonstrate how interactions between functional traits shape fitness and life history strategies. *Journal of Ecology*, 107, 2317–2328.
- QGIS Development Team. (2017). *QGIS geographic information system*. Open Source Geospatial Foundation Project.
- R Core Team. (2019). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from <http://www.R-project.org/>
- Roose, A., Kull, A., Gauk, M., & Tali, T. (2013). Land use policy shocks in the post-communist urban fringe: A case study of Estonia. *Land Use Policy*, 30, 76–83.
- Saar, L., Takkis, K., Pärtel, M., & Helm, A. (2012). Which plant traits predict species loss in calcareous grasslands with extinction debt? *Diversity and Distributions*, 18, 808–817.
- Shao, J.-W., Wang, H.-F., Fang, S.-P., Conti, E., Chen, Y.-J., & Zhu, H.-M. (2019). Intraspecific variation of self-incompatibility in the distylous plant *Primula merrilliana*. *Annals of Botany*, 11, plz030.
- Silvertown, J. (2009). A new dawn for citizen science. *Trends in Ecology & Evolution*, 24, 467–471.
- Steffan-Dewenter, I., & Westphal, C. (2008). The interplay of pollinator diversity, pollination services and landscape change. *Journal of Applied Ecology*, 45, 737–741.
- Stevens, C. J., Dise, N. B., Mountford, J. O., & Gowing, D. J. (2004). Impact of nitrogen deposition on the species richness of grasslands. *Science*, 303, 1876–1879.
- Sushinsky, J. R., Rhodes, J. R., Possingham, H. P., Gill, T. K., & Fuller, R. A. (2013). How should we grow cities to minimize their biodiversity impacts? *Global Change Biology*, 19, 401.
- Turrini, T., Dörler, D., Richter, A., Heigl, F., & Bonn, A. (2018). The threefold potential of environmental citizen science – Generating knowledge, creating learning opportunities and enabling civic participation. *Biological Conservation*, 225, 176–186.
- Valiente-Banuet, A., Aizen, M. A., Alcántara, J. M., Arroyo, J., Cocucci, A., Galetti, M., ... Zamora, R. (2015). Beyond species loss: The extinction of ecological interactions in a changing world. *Functional Ecology*, 29, 299–307.
- Van Rossum, F., Campos De Sousa, S. C., & Triest, L. (2004). Genetic consequences of habitat fragmentation in an agricultural landscape on the common *Primula veris*, and comparison with its rare congener, *P. vulgaris*. *Conservation Genetics*, 5, 231–245. <https://doi.org/10.1023/B:COGE.0000030007.85492.70>
- Van Rossum, F., De Sousa, S. C., & Triest, L. (2006). Morph-specific differences in reproductive success in the distylous *Primula veris* in a context of habitat fragmentation. *Acta Oecologica*, 30, 426–433.
- Van Rossum, F., & Triest, L. (2007). Fine-scale spatial genetic structure of the distylous *Primula veris* in fragmented habitats. *Plant Biology*, 9, 374–382.
- WallisDeVries, M. F., Poschlod, P., & Willems, J. H. (2002). Challenges for the conservation of calcareous grasslands in northwestern Europe: Integrating the requirements of flora and fauna. *Biological Conservation*, 104, 265–273.
- Watson, C. J., Carignan-Guillemette, L., Turcotte, C., Maire, V., & Proulx, R. (2019). Ecological and economic benefits of low-intensity urban lawn management. *Journal of Applied Ecology*, 57, 436–446. <https://doi.org/10.1111/1365-2664.13542>
- Wedderburn, F., & Richards, A. J. (1990). Variation in within-morph incompatibility inhibition sites in heteromorphic *Primula* L. *New Phytologist*, 116, 149–162.
- Wilson, J. B., Peet, R. K., Dengler, J., & Pärtel, M. (2012). Plant species richness: The world records. *Journal of Vegetation Science*, 23, 796–802.
- Yuan, S., Barrett, S. C. H., Duan, T., Qian, X., Shi, M., & Zhang, D. (2017). Ecological correlates and genetic consequences of evolutionary transitions from distyly to homostyly. *Annals of Botany*, 120, 775–789.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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